

June 27, 1937.

Dear Curt:

I am returning the paper which you sent to me. Since you stated you were leaving July 1, I have had to hurry to get it back to you in time and have not given it the amount of thought I should like to have. However, I have some comments which I should like to make.

The facts about the Wx - wx case of dimorphism of the pollen and its staining reaction is common knowledge to maize geneticists, i.e., when the reaction can be noted. From our experience the starch begins to appear shortly after the first division of the microspore (about 5 days after meiosis) but the staining reaction with iodine is not good until just before the pollen is ready to shed. The wx gene is not a good case since wx is located close to the spindle fiber attachment region on the short arm of chromosome 9 and probably is segregated in the first meiotic mitosis. The tetrads in higher plants are of two kinds with regard to division of the cytoplasm. In some (monocots mainly) the first mitosis is followed by cell wall formation. In other plants (dicots mainly) the two meiotic mitoses take place in a common cytoplasm. Wall formation does not occur until after the two nuclear divisions are completed. Datura has this form of tetrad production while maize has the former type.

The case Miss Clark is working on, which she has described to you, is quite relevant. At the end of the first meiotic mitosis 10 chromosomes (full set) are in each cell although not all in one nucleus. In the second division these nuclei form one to several spindles all in one cytoplasmic mass of each dyad. Wall formation takes place producing four or more cells depending upon the orientation of the spindles in the second division dyads. In each cell there are from one to several nuclei with one or more chromosomes in each. About five days elapse between the end of the meiotic divisions and the first division in the microspore in which growth and differentiation of the spores take place. What takes place during this period is a direct expression of the chromosome content of the nuclei. The spores with deficient chromosome complements do not develop normally (cytoplasm degenerates or nuclei appear degenerate) although they came from cytoplasm exposed during the second meiotic mitoses to the full genomic complement on the basis of release of genes at this period. The spores with a full genomic complement, although in separate nuclei, develop normally. In this case, the effect of the chromosome complement in the "resting nucleus" is immediately expressed. If genes were being released into the cytoplasm during division all the spores, regardless of their compositions, should progress normally until after the first division in the spore, unless the genes have been unequally distributed in the cytoplasm.

(over)

I don't know why they are in this position.

The spindle fiber is always attached to the chromosome.

If genes were released during division of the nucleus how would they be distributed to the cytoplasm of the two daughter cells?

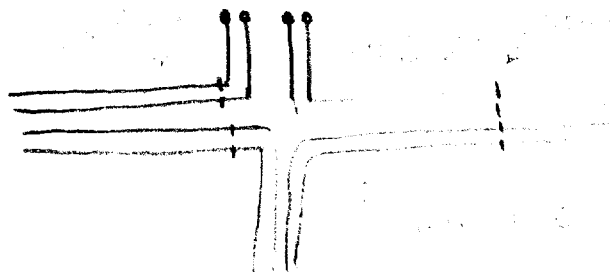
Cell wall formation takes place very rapidly in plants. It starts during anaphase + is completed by early telophase.

If genes were released here, some mechanism for distribution in the cytoplasm before wall formation or union of cytoplasm of adjacent cells, would have to be hypothesized. We do not get any spreading of genetic activity thru walls in the case of the PL gene which produces a purple cell sap whereas the other is a colorless sap. I have tested this with a ring of cells with PL when lost from the nucleus during mitotic divisions only PL cells develop. There is a spread of the Bm_1 gene (allele, Bm_1 , produces a brown cell wall).

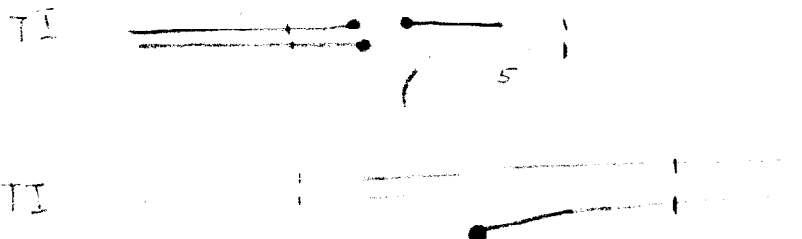
In a cell with Bm_1 which is adjacent to a Bm_2 cell, the wall immediately adjacent to the Bm_1 is Bm_1 or a very diluted Bm_1 . The Bm_1 effect spreads into the cell not only on the contact wall.

It seems to me release of genes at mitosis introduces another problem. You have not mentioned this in four papers.

There are other cases in maize that can be used. We have an interchange between chromosome 6 (with the nucleolus organizer) and chromosome 5. In the heterozygous condition it produces the following figure:



Crossing over occurs in the marked region in from 50 - 75% of the sporocytes (depending upon environmental conditions). As a result of such crossing-over, each telophase I nucleus has a full genomic complement (when such crossing-over occurs, segregation of homologous spindle fiber regions occurs always at I). At TI, the chromosome constitutions after such crossing-over are:



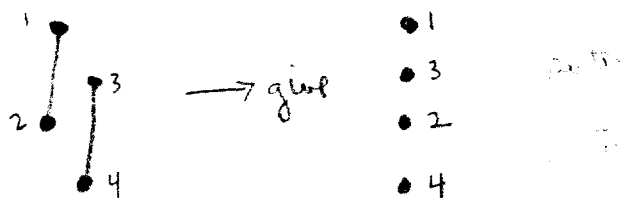
A full genomic complement is present in each nucleus, although not balanced. If genes are released during nuclear divisions, deficient and normal chromosome carrying spores should develop at similar rates.. This, however, does not occur. Spores with the full genomic complement develop much faster. Spores with ~~the~~ deficient chromosome complements develop much slower. I have not figured the rates of differential development of each type of spore in this particular interchange, where crossing-over has been measured, but it is a common practice to look for these types in ~~the deficient spores~~ anthers of different ages. The first spores to go through divisions are normal chromosome-carrying spores, the last to go through the first division in the spore are the deficient chromosome carrying spores. I can look more carefully for timing and let you know.

I think the spore development cases in maize could be used quite effectively if the right set-ups were chosen. I have several cases involving inversions and deficiencies which could be easily used. I will briefly outline a deficiency case which can be done easily. We have plants with a deficiency in chromosome 5 which is covered by a ring chromosome. Plants heterozygous for this deficiency and ring have one normal chromosome 5, one deficient chromosome 5 and a ring chromosome. The deficient and the normal chromosome 5 pair and segregate normally at meiosis.

The ring chromosome divides at I and gets into practically every TI nucleus. In II, the ring chromosome does not divide again but wanders to one or the other pole, sometimes being included in the nucleus and sometimes not. All the necessary genes are present in the TI nuclei but the TII nuclei are hyperploid, hypoploid and normal. The hypoploid pollen is practically empty of starch. I may be able to find a dimorphism of the spores before the first division and relate it to the deficient and full genomes carrying complements (the hyperploid complement is known to have practically the same division rate as the normal and need not be a factor). Since at metaphase II, the full genomic complement is present in all cells, there should be no spore dimorphism related to full and deficient genomic carriers on the basis of release of genes at nuclear divisions. If such is found, the constitution of the "resting nucleus" is the determining factor in differentiation.

I have spent some time describing the possibilities of spores in maize since I think they can be decisive cases. They can give better evidence than the wx gene. The case of endosperm constitution in Jone's paper is not particularly good in my estimation since he has not proven the number of nuclei in his endosperm cells. Some of his large cells may be multinucleate (I strongly suspect).

Is it obvious in Ustilago that the spores 1,2 and 3,4 are sister cells? In some forms the divisions are not strictly linear but the spindles are placed as follows:



In this case, adjacent spores are not sister spores.

The case of Pascher appears to me to be the strongest but I wish it were better analysed from a genetic point of view.

It seems to me that the geneticists have assumed that the genic activity of the nucleus takes place from the "resting nucleus" without, as you point out, the necessary evidence. I don't think our apparent evidence at the present time is very decisive. The geneticists have considered the "resting nucleus" as the actively functioning organ for so long, based on differentiation, dedifferentiation and wound responses (in plants) that they have not been alert to the evidence when it did appear and have not pointed it out as such but have taken it for granted.

You don't mention the paper of Berril and Huskins in the American Naturalist 70, (May-June) 1936, entitled "The Resting nucleus". You may have seen it and found it not to your point.

257-261

About publishing the paper - A short paper pointing out the need of decisive evidence is not out of order. A question for a title is quite appropriate under the circumstances. There should be some response to such a question. Would you publish in the Am. Nat.? It is an appropriate place for such an article.

I was pleased to learn you were to make a trip home. I hope it will be successful. Miss Clark tells me you are expecting another F_1 - or should'nt I know? I'll try to get up and see you after your return - sometime toward the end of August. Last summer was so difficult I had no time off all summer and was even three weeks late getting to my new job.

My best to you and Evelyn.

Sincerely,

Bar.

I am very this is no barely written + convinced, but there may be some thing in it you might wish to know, noticed I have made it any where near clear enough + I have my doubts!